

WHAT IS CLAIMED IS:

1. A DNA segment encoding a MURF-1, MURF-2 or MURF-3 polypeptide.
- 5 2. The DNA segment of claim 1, wherein the MURF-1, MURF-2 or MURF-3 polypeptide is murine.
3. The DNA segment of claim 2, wherein the MURF-1 polypeptide has the sequence of SEQ ID NO:2, the MURF-2 polypeptide has the sequence of SEQ ID NO:4, and the MURF-3 polypeptide has the sequence of SEQ ID NO:6.
- 10 4. The DNA segment of claim 3, wherein the MURF-1 DNA segment has the sequence of SEQ ID NO:1, the MURF-2 DNA segment has the sequence of SEQ ID NO:3, and the MURF-3 DNA segment has the sequence of SEQ ID NO:5.
- 15 5. The DNA segment of claim 1, wherein the DNA segment is positioned under the control of a promoter.
- 20 6. The DNA segment of claim 5, wherein the promoter is not a native MURF-1, MURF-2 or MURF-3 coding region.
- 25 7. The DNA segment of claim 5, wherein the MURF-1, MURF-2 or MURF-3 coding region is positioned in reverse orientation to the promoter, thereby capable of expressing an antisense product.
8. The DNA segment of claim 5, further comprising a polyadenylation signal.
9. The DNA segment of claim 5, further comprising an origin of replication.
- 30 10. The DNA segment of claim 9, wherein the vector is a viral vector.

11. The DNA segment of claim 10, wherein the vector is a non-viral vector.
12. A host cell comprising a DNA segment that encodes a MURF-1, MURF-2 or MURF-3 polypeptide, wherein said DNA segment comprises a promoter heterologous to the MURF-1, MURF-2 or MURF-3 coding region.
13. The host cell of claim 12, further defined as a prokaryotic host cell.
14. The host cell of claim 12, further defined as a eukaryotic host cell.
15. The host cell of claim 12, wherein the MURF-1, MURF-2 or MURF-3 polypeptide is murine.
16. The host cell of claim 14, wherein the host cell is a secretory cell.
17. The host cell of claim 15, wherein the MURF-1 polypeptide has the sequence of SEQ ID NO:2, the MURF-2 polypeptide has the sequence of SEQ ID NO:4, and the MURF-3 polypeptide has the sequence of SEQ ID NO:6.
18. A method of using a host cell comprising an expression cassette comprising a polynucleotide encoding a MURF-1, MURF-2 or MURF-3 polypeptide and a promoter active in said host cell, said promoter directing the expression of said polypeptide, said method comprising culturing the host cell under conditions suitable for the expression of the MURF-1, MURF-2 or MURF-3 polypeptide.
19. An isolated nucleic acid segment comprising at least 15 contiguous nucleotides of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5.
20. The isolated nucleic acid segment of claim 19, wherein said segment is 15 nucleotides in length.

- 5
- 10
- 15
- 20
- 25
- 30
21. The isolated nucleic acid segment of claim 19, wherein said segment is 20 nucleotides in length.
22. The isolated nucleic acid segment of claim 19, wherein said segment is 25 nucleotides in length.
23. The isolated nucleic acid segment of claim 19, wherein said segment is 30 nucleotides in length.
24. The isolated nucleic acid segment of claim 19, wherein said segment is 35 nucleotides in length.
25. The isolated nucleic acid segment of claim 19, wherein said segment is 50 nucleotides in length.
26. The isolated nucleic acid segment of claim 19, wherein the number of contiguous nucleotides is 20.
27. The isolated nucleic acid segment of claim 19, wherein the number of contiguous nucleotides is 25.
28. The isolated nucleic acid segment of claim 19, wherein the number of contiguous nucleotides is 30.
29. The isolated nucleic acid segment of claim 19, wherein the number of contiguous nucleotides is 35.
30. The isolated nucleic acid segment of claim 19, wherein the number of contiguous nucleotides is 50.

0900888 071201  
"02T20" 88680660

31. An isolated nucleic acid segment of from 14 to about 888 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, or complements thereof, under standard hybridization conditions.
- 5 32. The isolated nucleic acid segment of claim 31, further comprising an origin of replication.
33. The isolated nucleic acid of claim 31, wherein said isolated nucleic acid is a viral vector selected from the group consisting of retrovirus, adenovirus, herpesvirus, vaccinia virus, poxvirus, and adeno-associated virus.
- 10 34. The isolated nucleic acid of claim 31, wherein said nucleic acid is packaged in a virus particle.
35. The isolated nucleic acid of claim 31, wherein said nucleic acid is packaged in a liposome.
- 15 36. A nucleic acid detection kit comprising, in suitable container means, an isolated nucleic acid segment that hybridizes under high stringency conditions to the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, or complements thereof.
- 20 37. The kit of claim 36, further comprising a detection reagent.
- 25 38. The kit of claim 36, wherein said detection reagent is a detectable label that is linked to said nucleic acid segment.
39. The kit of claim 36, wherein the nucleic acid segment comprises a contiguous sequence from SEQ ID NO:1, SEQ ID NO:3 or SEQ ID NO:5, or complements thereof.
- 30

0950888 071804

40. The kit of claim 36, wherein the kit comprises pair of primers for amplifying a sequence from SEQ ID NO:1, SEQ ID NO:5 or SEQ ID NO:5, or complements thereof.
- 5 41. A composition comprising a purified MURF-1 or MURF-2 protein or peptide that includes a contiguous amino acid sequence from SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.
- 10 42. A purified MURF protein having the amino acid sequence of SEQ ID NO:2, SEQ ID NO: 4 or SEQ ID NO:6.
- 15 43. A recombinant MURF-1, MURF-2 or MURF-3 protein or peptide prepared by expressing a DNA segment that encodes a MURF-1, MURF-2 or MURF-3 protein or peptide in a recombinant host cell and purifying the expressed MURF-1, MURF-2 or MURF-3 protein or peptide away from total recombinant host cell components.
- 20 44. An isolated peptide of between about 10 and about 50 amino acids in length, comprising a contiguous amino acid sequence from the sequence of SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.
- 25 45. The peptide of claim 44, wherein said peptide is about 10 amino acids in length.
46. The peptide of claim 44, wherein said peptide is about 15 amino acids in length.
47. The peptide of claim 44, wherein said peptide is about 20 amino acids in length.
48. The peptide of claim 44, wherein said peptide is about 25 amino acids in length.
- 30 49. The peptide of claim 44, wherein said peptide is about 30 amino acids in length.

5

10

15

20

25

30

50. The peptide of claim 44, wherein said peptide is about 50 amino acids in length.
51. An antibody composition that binds to a protein or peptide that includes an epitope from SEQ ID NO:2, SEQ ID NO:4 or SEQ ID NO:6.
52. The antibody composition of claim 51, wherein the antibody composition comprises monoclonal antibodies.
53. The antibody composition of claim 51, wherein antibodies of the composition are operatively attached to a detectable label.
54. The antibody composition of claim 53, wherein the label is selected from the group consisting of a fluorescent label, a chemiluminescent label, a electroluminescent label, a radiolabel and an enzyme.
55. The antibody composition of claim 51, wherein the antibody composition is polyclonal.
56. A hybridoma cell that produces a monoclonal antibody that binds immunologically to MURF-1, MURF-2 or MURF-3.
57. An immunodetection kit comprising, in suitable container means, a first antibody that binds to a MURF-1, MURF-2 or MURF-3 protein or peptide.
58. The kit of claim 57, wherein the first antibody comprises is a detectable label.
59. The kit of claim 57, further comprising a second antibody that has binding affinity for the first antibody, the second antibody comprising a detectable label.
60. The kit of claim 57, wherein the first antibody is bound to a solid support.

10  
15  
20  
25  
30

61. A method for detecting alterations in MURF-1, MURF-2 or MURF-3 function in a cell comprising assessing the structure or expression level of a MURF-1, MURF-2 or MURF-3 polypeptide.
62. The method of claim 61, wherein assessing comprises determining the structure of a MURF-1, MURF-2 or MURF-3 gene.
63. The method of claim 62, comprising sequencing a MURF-1, MURF-2 or MURF-3 gene.
64. The method of claim 62, comprising Southern or Northern analysis of a MURF-1, MURF-2 or MURF-3 transcript or gene.
65. The method of claim 61, wherein assessing comprises determining the level of a MURF-1, MURF-2 or MURF-3 protein or transcript in the cell.
66. The method of claim 65, comprising Northern analysis of MURF-1, MURF-2 or MURF-3 transcripts.
67. The method of claim 65, comprising immunodetection of MURF-1, MURF-2 or MURF-3 protein levels.
68. The method of claim 67, wherein immunodetection comprises ELISA.
69. The method of claim 67, wherein immunodetection comprises Western blot.
70. A method for increasing MURF-1, MURF-2 or MURF-3 activity in cell comprising administering to the cell with an expression construct comprising a MURF-1, MURF-2 or MURF-3 coding region under the control of a promoter active in the cell.

71. The method of claim 70, wherein the promoter is myosin light chain-2 promoter, alpha actin promoter, troponin 1 promoter,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger promoter, dystrophin promoter, creatine kinase promoter, alpha7 integrin promoter, brain natriuretic peptide promoter, alpha B-crystallin/small heat shock protein promoter, alpha myosin heavy chain promoter and ANF promoter.
72. The method of claim 70, wherein the host cell is cardiomyocyte.
73. The method of claim 70, wherein the expression construct is a viral expression construct.
74. The method of claim 73, wherein the viral expression construct is encapsulated in a viral particle.
75. The method of claim 73, wherein the viral expression construct is selected from the group consisting of retrovirus, adenovirus, adeno-associated virus, herpesvirus, polyoma virus, vaccinia virus, and poxvirus.
76. The method of claim 70, wherein the expression construct is a non-viral expression construct.
77. The method of claim 76, wherein said expression construct is encapsulated in a liposome.
78. A method of screening a candidate substance for MURF-1, MURF-2 or MURF-3 binding activity comprising:
- (i) providing a MURF-1, MURF-2 or MURF-3 polypeptide;
  - (ii) contacting the MURF-1, MURF-2 or MURF-3 polypeptide with the candidate substance; and



- (iii) determining the binding of the candidate substance to the MURF-1, MURF-2 or MURF-3 polypeptide.

79. The method of claim 78, wherein the assay is performed in a cell free system.

80. The method of claim 78, wherein the assay is performed in a cell.

81. The method of claim 78, wherein the assay is performed *in vivo*.

82. A method of screening a candidate substance for an effect on MURF-1, MURF-2 or MURF-3 levels in a cell comprising:

- (i) providing a cell that expresses MURF-1, MURF-2 or MURF-3 polypeptide;
- (ii) contacting the cell with the candidate substance; and
- (iii) determining the effect of the candidate substance on MURF-1, MURF-2 or MURF-3 polypeptide level.

83. A method of screening a candidate substance for an effect on MURF-1, MURF-2 or MURF-3 expression in a cell comprising:

- (i) providing a cell that expresses MURF-1, MURF-2 or MURF-3 polypeptide;
- (ii) contacting the cell with the candidate substance; and
- (iii) determining the effect of the candidate substance on MURF-1, MURF-2 or MURF-3 mRNA levels.

84. A method of screening a candidate substance for an effect on MURF-1, MURF-2 or MURF-3 interaction with microtubules comprising:

- (i) providing a microtubule composition;

- (ii) contacting the microtubule composition with MURF-1, MURF-2 or MURF-3 polypeptide in the presence of the candidate substance; and
- (iii) assessing the interaction of MURF-1, MURF-2 or MURF-3 with the microtubule composition in the presence of the candidate substance,

wherein a change in the interaction of MURF-1, MURF-2 or MURF-3 with the microtubule composition, as compared to the interaction in the absence of the candidate substance, indicates that the candidate substance modulates the interaction of MURF-1, MURF-2 or MURF-3 and microtubules.

85. The method of claim 84, wherein step (ii) is performed in a cell free system.

86. The method of claim 84, wherein step (ii) is performed in a cell.

87. The method of claim 84, wherein step (ii) is performed *in vivo*.

88. The method of claim 84, wherein step (iii) comprises a cosedimentation assay.

89. A method for screening a candidate substance for an effect on MURF-1, MURF-2 or MURF-3 homodimerization comprising:

- (i) providing a MURF-1, MURF-2 or MURF-3 polypeptide composition;
- (ii) contacting the composition with the candidate substance; and
- (iii) determining the effect of the candidate substance on MURF-1, MURF-2 or MURF-3 homodimerization.

90. A method of screening a candidate substance for an effect on MURF-1, MURF-2 or MURF-3 directed glutamic acid modification of microtubules comprising:

- (i) providing a cell that expresses MURF-1, MURF-2 or MURF-3 polypeptide;

- (ii) contacting the cell with the candidate substance; and
- (iii) determining the effect of the candidate substance on glutamic acid modification of microtubules.

5 91. A method of screening a candidate substance for an effect on MURF-1, MURF-2 or MURF-3 stabilization of microtubules comprising:

- (i) providing a microtubule composition;
- (ii) contacting the microtubule composition with MURF-1 or MURF-2 polypeptide in the presence of the candidate substance; and
- (iii) assessing the stability of the microtubule composition in the presence of the candidate substance,

10  
15 wherein a change in the stability of MURF-1, MURF-2 or MURF-3 with the microtubule composition, as compared to the stability in the absence of the candidate substance, indicates that the candidate substance modulates the stability of microtubules.

20 92. A transgenic non-human mammal, cells of which comprise a MURF-1, MURF-2 or MURF-3 encoding nucleic acid segment integrated into their genome, wherein the MURF-1, MURF-2 or MURF-3 encoding nucleic acid is under the control of a heterologous promoter.

25 93. The transgenic mammal of claim 92, wherein the promoter is a tissue specific promoter.

94. The transgenic mammal of claim 93, wherein the tissue specific promoter is a muscle specific promoter.

30 95. The transgenic mammal of claims 94, wherein the muscle specific promoter is selected from the group consisting of myosin light chain-2 promoter, alpha actin

promoter, troponin 1 promoter,  $\text{Na}^+/\text{Ca}^{2+}$  exchanger promoter, dystrophin promoter, creatine kinase promoter,  $\alpha 7$  integrin promoter, brain natriuretic peptide promoter, myosin heavy chain promoter, ANF promoter, and  $\alpha$  B-crystallin/small heat shock protein promoter.

5

96. The transgenic mammal of claim 92, wherein said mammal is a mouse.

10

97. A method of treating cardiac failure comprising increasing MURF-1, MURF-2 or MURF-3 activity in a cardiac cell, wherein said increased MURF-1, MURF-2 or MURF-3 activity stabilizes microtubules and/or intermediate filaments.

15

98. The method of claims 97, wherein increasing MURF-1, MURF-2 or MURF-3 activity comprises contacting said cardiac cell with an expression cassette that comprises a polynucleotide encoding a MURF-1, MURF-2 or MURF-3 polypeptide and a promoter active in said cardiac cell, wherein said promoter directing the expression of said polypeptide.

20

99. The method of claim 98, wherein said promoter is a cardiac specific promoter.

100. The method of claim 98, wherein contacting comprises intravenous or intraarterial administration of a vector comprising said expression cassette.

25

101. A method of modulating MURF-1, MURF-2 or MURF-3 activity in a cell comprising administering to said cell an agent that modulates MURF-1, MURF-2 and/or MURF-3 activity.

102. The method of claim 101, wherein said agent inhibits MURF-1, MURF-2 or MURF-3 activity.

30

103. The method of claim 10, wherein said agent is a small molecule.

104. The method of claim 102, wherein said agent is an antisense molecule that hybridizes to MURF-1, MURF-2 and/or MURF-3 transcripts.
105. The method of claim 102, wherein said agent is a ribozyme molecule that cleaves MURF-1, MURF-2 and/or MURF-3 transcripts.
106. The method of claim 101, wherein said agent enhances MURF-1, MURF-2 or MURF-3 activity.
107. A method of blocking MURF-1, MURF-2 or MURF-3 expression in a cell comprising administering to said cell an agent that inhibits transcription or translation of MURF-1, MURF-2 and/or MURF-3.
108. A method of increasing MURF-1, MURF-2 or MURF-3 expression in a cell comprising administering to said cell an agent that promotes transcription or translation of MURF-1, MURF-2 and/or MURF-3.
109. A method of screening a candidate substance for an effect on MURF-1, MURF-2 or MURF-3 interaction with intermediate filaments comprising:
- (i) providing an intermediate filament composition;
  - (ii) contacting the intermediate filament composition with MURF-1, MURF-2 or MURF-3 polypeptide in the presence of the candidate substance; and
  - (iii) assessing the interaction of MURF-1, MURF-2 or MURF-3 with the intermediate filament composition in the presence of the candidate substance,

wherein a change in the interaction of MURF-1, MURF-2 or MURF-3 with the intermediate filament composition, as compared to the interaction in the absence of the candidate substance, indicates that the candidate substance modulates the interaction of MURF-1, MURF-2 or MURF-3 and intermediate filament.

5

110. The method of claim 109, wherein step (ii) is performed in a cell free system.

111. The method of claim 109, wherein step (ii) is performed in a cell.

112. The method of claim 109, wherein step (ii) is performed *in vivo*.

113. The method of claim 109, wherein step (iii) comprises a cosedimentation assay.

10

114. The method of claim 109, wherein said intermediate filaments are one or more of desmin, vimentin and cytokeratin.

15

115. A method for screening a candidate substance for an effect on MURF heterodimerization comprising:

20

- (i) providing two or more of a MURF-1, MURF-2 or MURF-3 polypeptide composition;
- (ii) contacting the compositions with the candidate substance; and
- (iii) determining the effect of the candidate substance on the heterodimerization of two or more of MURF-1, MURF-2 or MURF-3.

25